The Effects of Light Color on Performance and Immune Response of Broiler Chickens

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Abstract

Environmental factors in poultry housing, such as crowding, temperature and lighting, influence health and productivity of chickens. Lighting is an important factor in regulating physiological and behavioral processes in chickens. Different wavelengths of light have shown to affect growth rate and immune response in broiler chickens and egg quality in layer hens. Laying hens exposed to red light have increased egg production and broilers exposed to blue and green lights have increased weight gain. Previous research has studied the effects of different colored lighting on the immune responses in chickens vaccinated for Infectious Bursal Disease and Newcastle Disease; however, no research thus far has studied chickens vaccinated against Marek's Disease under these conditions. In the present study one hundred and twelve day-of-hatch broilers were reared under white, blue, green and red LED bulbs with an intensity of 15 lux and a 23L:1D photoperiod. Chicks were vaccinated subcutaneously with a commercial Marek's Vaccine on day of hatch. Birds were weighed and blood samples collected on d 0, 7, 14, 21, 28, 35, 42 and 49. Total serum IgY concentrations were increased in broilers raised under red light treatment on d 35 and 42. Blue light and green light treatment resulted in the highest and lowest body weight of birds, respectively, on d 42 and 49.

Introduction

The transition from barnyard to commercial poultry production has resulted in the rearing of chickens in large barns or brooder houses which require intensive management. To facilitate production, producers are capable of manipulating and modulating environmental parameters such as temperature, humidity, ventilation, gases, light intensity, light duration and light color. Of these factors, light may be the most critical for chickens as it controls many physiological and behavioral processes (Olanrewaju *et al*., 2006). Light allows for the establishment of circadian rhythms and synchronization of various essential physiological functions including body temperature, metabolism and secretion of hormones that influence growth, maturation and reproduction (Manser, 1996). The lighting program for a particular brooder house varies depending on the type of poultry (layer hen, breeding stock vs. broiler, turkey) and even the specific genetic strain of poultry.

The impact light has on both avian physiology and behavior is largely due to three factors: intensity, photoperiod (i.e. the duration of exposure to light) and wavelength. Light intensity is traditionally measured in luminous flux (lux) and can have a significant impact on the health and welfare of poultry. For example, white light intensity that is 1 lux or higher is bright enough to stimulate a light-dark cycle in broiler chickens with higher intensities showing a more noticeable circadian rhythm (Blatchford *et al.,* 2012). The intensity of light under which broilers are raised also affects resting behavior and activity. Broiler raised under 5 lux of light demonstrated more frequent resting bouts than those raised under 50 and 200 lux while birds raised under the latter conditions demonstrated higher activity during the photoperiod and longer resting bouts during the scotoperiod (Alvino *et al.,* 2009). The blood profiles of broilers reared under varying light intensities showed significant differences in cholesterol and thyroxin (T4) levels while cortisol, urea, creatinine, triiodothyronine, respiratory rate and rectal temperature were not different

(Mahmood, *et al.*, 2014). Blatchford and colleagues (2009) found that birds raised under 200 lux intensity had more bruising and fewer erosions on the hocks and footpads compared to birds raised under less light intensities, as well as birds raised at 50 lux having an increase in the primary IgM response to a novel antigen. Abbas *et al*. (2014) found broilers raised under 5 lux had significantly higher antibody titers against Infectious Bursal Disease compared to birds raised under 10, 20, 30 and 40 lux.

The photoperiod in a lighting program can also have varying effects on performance, behavior and physiological indices. In leghorn cockerels immunized against sheep red blood cells (SRBC) there was a significant difference in antibody production between birds that were reared in a constant light period compared to those with a 12L:12D period (Kirby and Froman, 1991); however, broiler chickens immunized with SRBC reared under the same lighting conditions did not have a significant difference in antibody titers (Abbas *et al*., 2008). The length of photoperiod for male broilers was found to influence CD4+ and B-lymphocytes at 3 weeks of age and CD4+, CD8+ and CD3+ lymphocytes at 6 weeks of age (Kliger *et al*., 2000). Body weight has been found to be consistent among broilers reared under different photoperiods, though Schwean-Lardner and colleagues (2012) found that broilers reared under 17 and 20 hr of light attained the heaviest body weights compared to broilers raised under 14 and 23 hr. Similarly, broiler chickens were found to have decreased body weights when reared under a 12L:12D cycle compared to a near continuous 23L:1D cycle and an intermittent 2L:2D cycle (Abbas et al., 2008). Longer photoperiods reduced mobility, exploratory, comfort and nutritive behaviors in broilers, while a photoperiod of 17 hr produced maximum behavioral expression (Schwean-Lardner *et al.*, 2012).

Wavelength also has a critical impact on poultry production and physiology and has become a recent topic of interest. Domestic fowl have well-developed color vision that is superior to humans. They are capable of sensing ultraviolet, blue, green and red on the light spectrum

(Prescott and Wathes, 1999) via retinal and deep brain photoreceptors (Kuenzel *et al*., 2015). Laying hens exposed to red light have increased production (Pyrzak, *et al*., 1987; Hassan *et al*., 2013; Huber-Eicher *et al*., 2013; Hassan *et al*., 2014) but generally smaller egg size (Er *et al.*, 2007). When exposed to white, blue and green lighting, laying hens produced eggs that were heavier, rounder and higher in eggshell quality, respectively (Er *et al.*, 2007). Broilers reared under blue and green monochromatic light experienced increased body weights compared to broilers exposed to white or red light (Cao *et al*, 2012; Kim *et al*., 2013; Hassan *et al*, 2014). Exposure to different wavelengths also had an effect on immune responses with variability in humoral and cellular immune response between light treatments, day of age and vaccination status (Xie *et al*., 2008; Sadrzadeh *et al*., 2011; Firouzi *et al*., 2014; Zhang *et al*., 2014).

Recently, the effects of wavelength on the immune response of chickens vaccinated against Infectious Bursal Disease and Newcastle Disease have been studied and found to influence humoral and cellular immune responses (Xie *et al*., 2008; Sadrzadeh, *et al*., 2011; Zhang *et al*., 2014; Firouzi *et al*., 2014). Chickens vaccinated against Marek's Disease have not been studied under these lighting conditions. Marek's Disease is a common lymphoproliferative disease of chickens caused by a ubiquitous oncogenic herpes virus spread through feather dander. Infected birds exhibit signs of paralysis, decreased production and, upon necropsy, visceral tumors, all due to T-lymphocyte transformation (Nair, 2005; Baigent *et al*., 2006). Marek's Disease condemnation in chickens accounts for approximately US$1 billion (Nair, 2005). By manipulating the environmental factors such as light color in poultry housing, immune function could be enhanced to elicit a stronger response to pathogens that typically decrease productivity and jeopardize welfare. Thus, the objective of this study was to determine if light wavelength can affect performance and humoral immune response in broilers vaccinated against Marek's Disease.

Materials and Methods

One hundred and twelve day-of-hatch broiler chicks were obtained from a local commercial hatchery and randomly assigned to one of four light treatment groups [white (400 to 700 nm), blue (450-495 nm), green (495-570 nm) and red (620-700 nm)]. Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed *ad libitum*. On day of hatch (d0), all chicks were vaccinated subcutaneously with a commercial Marek's Disease vaccine according to the manufacturer's directions (Zoetis, Inc.; Florham Park, NJ). Each pen contained one brooder lamp with a light emitting diode (LED) bulb of its respective color equalized at an intensity of 15 lux measured at ground height and a photoperiod of 23L:1D. On d 7, 14, 21, 28, 35, 42 and 49, all birds were weighed to evaluate growth performance. Feed consumption was recorded daily to calculate feed conversion. In addition, blood samples were collected via jugular or brachial venipuncture from five birds per treatment group to evaluate IgY response to vaccination.

Serum samples were assayed for total immunoglobulin (Ig) Y concentrations using a commercial quantitative enzyme-linked immunosorbent assay (ELISA) kit (Bethyl Laboratories; Montgomery, TX). One µl of diluted anti-chicken IgG goat affinity antibody was diluted to 100 µl with coating buffer and 100 µl of the diluted coating antibody was then added to each well. After incubation for 1 hr at room temperature, the wells were washed five times with an ELISA wash solution, and 200 µl of blocking solution was added to each well. After a maximum incubation period of 24 hr at room temperature, the wells were washed with an ELISA wash solution and 100 µl of diluted sample and standard were added to each well. Serum sample dilutions ranged from 1:20,000 to 1:50,000. After incubation for 1 hr at room temperature, the wells were washed with an ELISA wash solution and 100 µl of diluted horse radish peroxidase (HRP) detection antibody was added to each well. After incubation for 1 hr at room temperature, the wells were washed with an ELISA wash solution and 100 µl of trimethylbenzidine (TBS)

was added to each well and allowed to incubate for 15 min. in a dark room. Lastly, 100 µl of stop solution consisting of H_3PO4 was added. The absorbance of each well was measured using an ELISA reader at an optical density (OD) of 450 nm. A 4-parameter analysis was used to set a standard curve for IgY values. Statistical analysis of the data was performed using SAS v9.3 software (SAS Institute, Inc.; Cary, NC).

Results

On d 0, 7, 14 and 21 there were no significant differences in body weight between treatment groups (Figure 1). On d 28 birds raised under red light weighed significantly less compared to birds raised under blue and white treatments ($P < 0.05$). On d 35, broilers raised under red light weighed significantly less than birds raised under blue and white treatments ($P < 0.05$). In addition, birds raised under blue light treatment weighed significantly more than birds raised under green light ($P < 0.05$). On d 42, birds raised under white and blue light weighed more than birds raised under green and red light, while birds raised under red light weighed more than birds raised under green light ($P < 0.05$). On d 49, body weight in broilers raised under blue light was greater than broilers raised under white, green and red light treatments ($P < 0.05$) Broilers raised under green light had significantly lower body weight compared to broilers raised under white and red light treatments ($P < 0.05$), though body weight did not significantly differ between broilers raised under white and red light.

Table 1 shows the feed conversion ratios (FCR) and average carcass weights (g) per treatment group. Birds raised under blue light had the highest FCR (1.82) and carcass weight while birds raised under green light had the lowest FCR (1.62) and carcass weight. Birds raised under red and white light treatments had similar FCR (1.65) and carcass weights.

There were no differences in IgY concentrations in response to vaccination between birds raised under the different light colors on d 7, 14, 21 and 49. IgY concentrations were significantly higher in the red treatment compared to the white treatment on d 28 ($P < 0.05$). On d 35 and 42, IgY concentrations of broilers reared under red lighting were greater compared to IgY concentrations of broilers reared under white, blue and green lighting ($P < 0.05$).

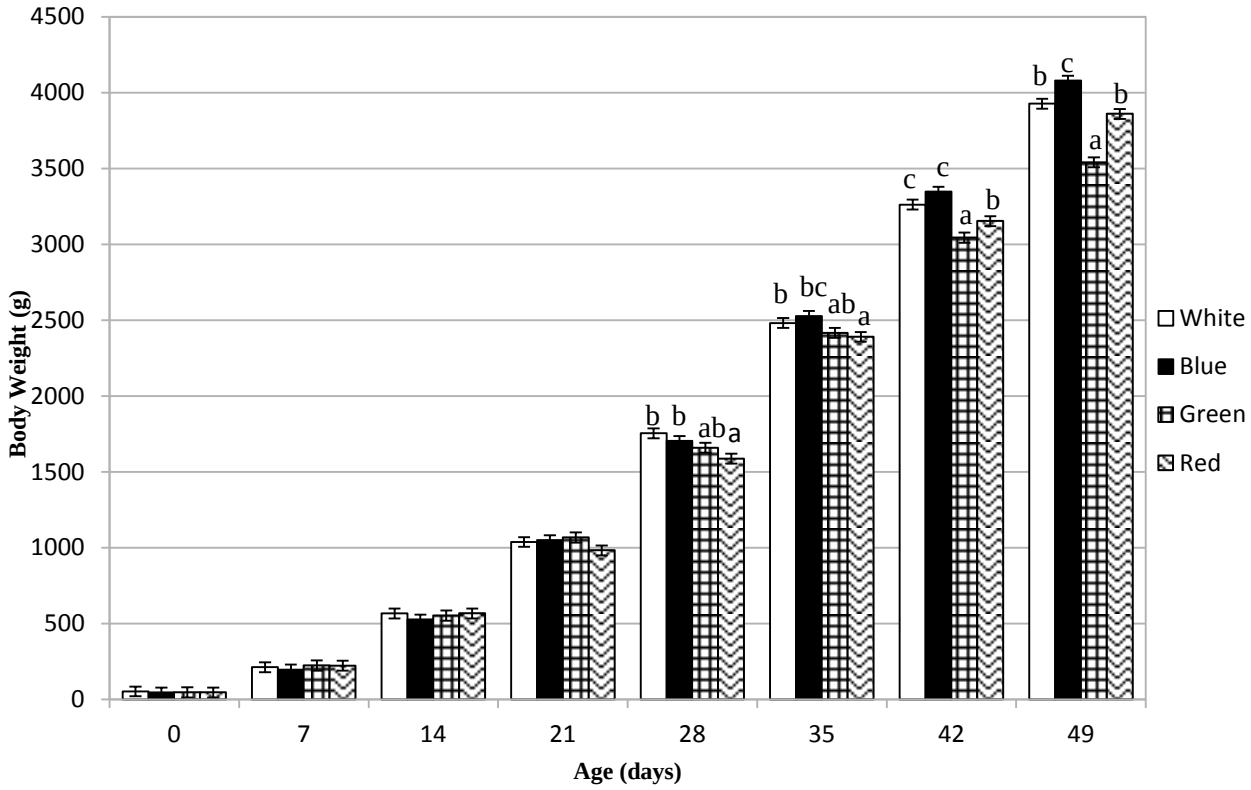

Figure 1: Live body weight (kg) of male broiler chicks (n=112) reared under different light wavelengths of white (400-700 nm), blue (450-495 nm), green (495-570 nm), and red light (620-700 nm) at 0, 7, 14, 21, 28, 35, 42 and 49 d of age. Values are represented as mean ± standard error. Means with no common superscript at the same time period differ significantly ($P < 0.05$).

Light Treatment	Feed Conversion Ratio (FCR)	Avg. Carcass Weight (g)
White	1.65	3,045 ± 227
Blue	1.82	3,150 ± 186
Green	1.62	2,745 ± 336
Red	1.65	2,950 ± 236

Table 1: Feed conversion ratio (FCR) and average carcass weights (g) of male broiler chicks (n=112) reared under different light wavelengths of white (400-700 nm), blue (450-495 nm), green (495-570 nm), and red light (620-700 nm). FCR was calculated by total feed consumed divided by average total live body weight gained per treatment group. Carcass weight values are represented as mean ± standard deviation.

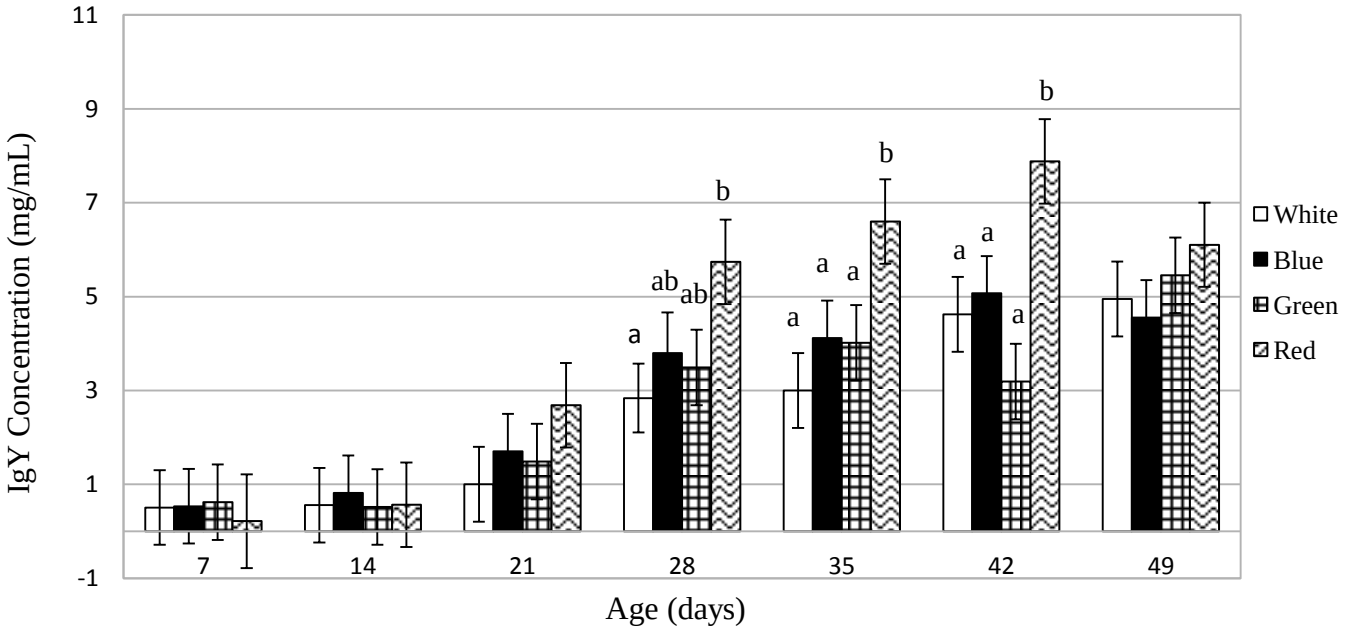

Figure 2: Serum IgY titers (mg/mL) of male broiler chicks reared under white (400-700 nm), blue (450-495 nm), green (495-570 nm), and red (620-700 nm) light at 7, 14, 21, 28, 35, 42 and 49 d of age. IgY concentrations were measured by quantitative ELISA at OD 450 nm. Values are represented as mean ± standard error. Means with no common superscript within day differ significantly ($P < 0.05$).

Discussion

The results of this study contrast those of previous research. In the present study, light color influenced the live body weight and carcass weights of broilers. Rozenboim *et al*. (2013) reported significant differences in body weight throughout the experimental period with broilers exposed to blue (480 nm) and green (560 nm) light gaining the most weight on d 25 and 34. In this study, birds raised under blue and white light gained the most body weight on d 28 and 35 compared to birds raised under green and red light. Other studies have also reported increased body weight in broilers reared under blue and green monochromatic light (Cao *et al*., 2012; Hassan *et al*., 2014). Although Firouzi *et al*. (2014) noted that broilers raised under green light were significantly heavier compared to other colors, broilers reared under blue light were the lightest of those evaluated. Conversely, broilers in this study attained the highest body weight in the blue light treatment group, and broilers in the green light treatment group gained the least amount of body weight. Feed conversion ratios (FCR) and carcass weights were positively correlated with live body weights for each treatment group.

Light color also influenced the immune response in the present study. Xie and colleagues (2008) found that broilers vaccinated for Newcastle Disease and reared under blue and green light maintained higher anti-NDV titers throughout the entire experimental period. After the first vaccination, birds raised under green light had greater anti-NDV titers compared to birds raised under different light conditions on d 14, though this difference was not significant. The broilers raised under green light treatment also showed significant increases in antibody titers compared to the broilers raised under red light treatment on 28 after the second vaccination. However, on d 42 and 49 the broilers raised under the blue light treatment had the highest antibody titer. Zhang *et al*. (2014) found similar results with anti-NDV and anti-BSA titers highest in the green light treatment while antibody titers for broilers in the white and red light treatment groups remained similar throughout the experiment. Recently, reports from Firouzi and colleagues (2014) differed

slightly. On d 18 and 42 anti-NDV titers in the green treatment were higher than other treatments, though the blue light treatment showed higher concentrations on d 30 and red light maintained the lowest titers on d 18, 30 and 42 (Firouzi *et al.*, 2014). Serum IgY (referred to as IgG) concentrations were studied by Hassan and colleagues (2014) in broiler chicks only in blue, green and white light treatments on d 21 and 35. They reported green and blue light resulted in higher immunoglobulin concentrations on d 21 and 35, respectively.

Difference in methodology between this study and those previously discussed may account for the differences in results. Photoperiod, intensity, stocking density, light source, light height, broiler sex, sample size and light wavelength were not believed to be responsible for differing results; however, broiler strain, immunoglobulin specificity, sampling day and vaccination may explain differences observed. Certain genetic lines of poultry are capable of differing in their immunological responsiveness to pathogens (Yonash *et al*., 1996; Kaiser *et al*., 1998; Yang *et al*., 2000). Because of the unknown genetic composition of the broilers in this study, their genetic contribution to immune response cannot be determined or compared to the strains studied in previous research. In addition, the aforementioned studies measured antibody titers specific to the vaccine antigen while this study measured total IgY. Total IgY could encompass antibodies specific to antigens other than the ones presented via the Marek's Disease vaccination. Virus pathology may also play a part, as the virus in Newcastle Disease vaccines targets different tissues (Wakamatsu *et al*., 2006) than the lymphotropic herpes virus of turkeys (HVT) in the Marek's Disease vaccine (Fabricant *et al*., 1981; Wakamatsu *et al*., 2006). Other research suggests that the differences noted are due to induction of B-lymphocyte proliferation via stimulation of pineal melatonin secretion from exposure to green monochromatic light (Li *et al*., 2015).

In conclusion, the color of light increased the antibody response of broilers while not adversely affecting their performance. Broilers reared under blue light attained higher body

weight while those reared under green light attained the lowest. Broilers raised under red lighting had increased IgY titers than broilers raised under white, blue or green light. Therefore, red light may be applied in poultry production systems to enhance the immune responsiveness of broilers to vaccination without adversely affecting performance.

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